

## CLAIMS

1. A method for determination of concentration of one or more analytes in a test sample or an aliquot of a test sample of a complex biological fluid, characterised by
  - 5 a) mixing the said sample or aliquot of the said sample with one single reagent, such as a solid, a solution or premixed solution, wherein said reagent being provided in one single container or compartment of a container, and no other reagent is added during the performance of said method, and said reagent further comprises at least one type of binding molecule with specific affinity for one or more of the said analytes, and
  - 10 b) said reagent furthermore comprises either fluorescent moieties covalently linked to the said binding molecules or fluorescent analogues of or fluorescent fragments of or fluorescent derivatives of said analyte or analytes, and
  - 15 c) measuring the polarisation of the emitted light, and
  - d) calculating the concentration or concentrations of said analyte or analytes.
2. A method according to claim 1, characterised by using a reagent for each analyte comprising immunocomplexes between
  - 20 a) an antibody or an immunoactive fragment of an antibody with specific affinity for said analyte or analytes, and
  - b) fluorescent analogues or fluorescent fragments of or fluorescent derivatives of said analyte or analytes.
3. A method according to claim 1, characterised by using a reagent for each analyte comprising complexes between
  - 25 a) an aptamer or another synthetic binder with a specific affinity for said analyte, and
  - b) fluorescent analogues or fluorescent fragments of or fluorescent derivatives of said analyte or analytes.
4. A method according to claim 1, characterised by using a reagent comprising binding molecules with specific affinity for one or more of the said analytes and with fluorescent moieties with absorption maximum between 600 nm and 1000 nm, preferably above 620 nm, covalently linked to the said binding molecules, and said binding molecules being either a peptide or being synthetic binders, optionally being identified by combinatorial chemistry techniques or phage display or nucleic acid selection technology.
- 35 5. A method according to any of the claims 1 to 4, characterised by using a reagent comprising fluorescent binding molecules with

specific affinity for one analyte, or comprising fluorescent analogues of, or fluorescent fragments of, or fluorescent derivatives of one analyte only.

6. A method according to any of the claims 1 to 5,  
characterised by the use of a reagent comprising different fluorescent moieties  
5 covalently bound to different binding molecules with different specific affinities.
7. A method according to any of the claims 1 to 6,  
characterised by the use of a reagent comprising one or more peptides or derivatives  
of peptides with specific binding affinity for an analyte, said binding peptides having a  
fluorescent residue covalently linked and being constituted by less than 30 amino  
10 acids.
8. A method according to claim 7,  
characterised in that binding peptide is constituted by less than 20 amino acids.
9. A method according to claim 7,  
characterised in that binding peptide is constituted by less than 15 amino acids.
- 15 10. A method according to any of the claims 1 to 9,  
characterised by the use of a reagent comprising peptides or derivatives of peptides  
containing amino acid sequence Ala-Arg-Asn-Arg-Asn or Ala-Arg-Asn-Gly-Asn for  
quantitation of C-reactive protein.
11. A method according to any of the claims 1 to 10,  
20 characterised by the use of a reagent with fluorescent residues with maximum  
coefficient of absorption at a wavelength above 640 nm.
12. A method according to any of the claims 1 to 11,  
characterised by the use of a reagent comprising cell lysing substances or anti-  
coagulants or detergents.
- 25 13. A method according to any of the claims 1 to 12,  
characterised by the use of a reagent comprising one or more fluorescent moieties  
selected from the group consisting of fluoresceine, Texas Red, Cy5, other Cy Dye  
FluorLink substances, other Cyanin derivatives, Rhodamin, Methyl Rhodamin,  
Biodypi 630/650-X/MeOH, Biodypi 650/655-X/MeOH, Biodypi FL/MeOH, Biodypi  
R6G/MeOH, Biodypi TMR-X/MeOH Biodypi TR-X/MeOH or other substances from  
30 the Biodypi group of substances, Alexa Fluor Dyes of different wavelengths,  
Ruthenium ligand complexes, lanthanoid elements such as Europium, Samarium or  
Terbium complex bound to a chelating ligand like DTPA, EDTA or N1.
14. A method according to any of the claims 1 to 13,  
35 characterised by that the polarisation of the emitted light is measured as a function of

time, either as a continuous kinetic reading or a reading of the change in polarisation of the emitted light between two or more time points, or as a measurement of the polarisation of the emitted light after a defined point of time.

15. A method according to any of the claims 1 to 14,  
5 characterised by that sample material or aliquot of the sample material is constituted by a biological material, or a dilution or an extract or being dissolved from or being filtrated from the said biological material.
16. A method according to any of the claims 1 to 15,  
10 characterised by that sample material or aliquot of the sample material is constituted by blood, or blood serum, or blood plasma, or blood cells, or lysate from blood or blood cells, or urine, or cerebrospinal fluid, or tear liquid, or sputum, or semen, or plasma, or semen or material aspirated from the gastro-intestinal tract or feces, or extract or filtrate of suspension of feces, or plant material or extracts thereof, or dissolved plant material or filtrate thereof.
- 15 17. A method according to any of the claims 1 to 16,  
characterised by the use of standards or calibrators comprising known concentrations of the analyte or the analytes, and furthermore wherein the concentration or concentrations of said analyte or analytes in unknown samples is calculated by interpolation of the values obtained from the unknown samples on the standard curve  
20 obtained from said known standards or calibrators.
18. A method according to any of the claims 1 to 17,  
characterised by the use of a standard curve stored in an artificial memory, optionally connected to the fluorescent polarisation instrument in use.
- 25 19. A method according to any of the claims 1 to 18,  
characterised by the use of temperature correction algorithms, either generated empirically or theoretically, to compensate for differences in fluorescence polarisation caused by differences in temperature at different time of measurements of standards and unknown samples, or between standards, or between unknown samples.

20. A method according to any of the claims 1 to 19,  
characterised by being provided in concentrated or dry form, to be diluted or  
reconstituted before use, the said reagent being provided divided between different  
compartments for combination into one reagent prior to use.

5 21. A reagent for the performance of the method according to any of the claims 1 to  
20,  
characterised in that said reagent comprises at least one type of binding molecule  
with specific affinity for one or more of the said analytes, and said reagent  
furthermore comprises fluorescent moieties covalently linked to the said binding  
10 molecules or fluorescent analogues of or fluorescent fragments of or fluorescent  
derivatives of said analyte or analytes.

22. A reagent according to claim 21,  
characterised in that the reagent comprises complexes between  
a) an antibody or an immunoactive fragment of an antibody or an aptamer or a  
15 synthetic binder with specific affinity for at least one analyte and  
b) fluorescent analogues or fluorescent fragments of or fluorescent derivatives of said  
analyte or analytes.

23. A reagent according to claims 21 to 22,  
characterised in comprising binding molecules with specific affinity for one or more of  
20 the said analytes and optionally with fluorescent moieties with absorption maximum  
between 600 nm and 1000 nm, preferably exceeding 620 nm, more preferably  
exceeding 640 nm, covalently linked to the said binding molecules, and said binding  
molecules being either of peptide or aptamer composition or being synthetic binders,  
optionally being identified by combinatory chemistry techniques or phage display or  
25 nucleic acid selection technology.

24. A reagent according to claims 21 to 23,  
characterised in being an assay reagent comprising peptid binders or binders of  
derivatives of peptides, including fluorescent derivatives of said binders, containing the  
amino acid sequence Ala-Arg-Asn-Arg-Asn and/or Ala-Arg-Asn-Gly-Asn.

30 25. Use of the method according to claims 1 to 20 to determine concentrations of  
clinically related substances in samples of biological material from living organisms in  
need thereof.

26. Kit for the determination of concentration of one or more analytes in a test sample or an aliquot of a test sample of complex biological fluid,  
characterized in comprising one or more containers, wherein the container(s) or compartment of the container(s) contains one single reagent, preferably in the fluidal  
5 state and according to any of the claims 21-24, and wherein the reagent comprises  
one or more fluorescence-labelled specific binding molecules towards the analyte(s) to  
be measured, or a fluorescence-labelled analogue or a fluorescent fragment or a  
fluorescent derivative of said analyte(s), as well as device for obtaining the exact  
10 volume(s) of the complex biological fluid to be tested and that is needed in order to  
perform the method adequately.

27. Kit according to claim 26,  
characterized in that the reagent which is contained in a container or a compartment  
of a container, is formed to a ready-for-use reagent by mixing the content from  
different containers prior to or immediately prior to or in connection with the  
15 execution of the analysis.